Journal of Experimental Agriculture International

Volume 45, Issue 10, Page 59-74, 2023; Article no.JEAI.105181
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Development of a Hydrothermal Treatment to Control Fruit Flies (Diptera: Tephritidae) and Maintain the Quality of 'Fuyu' Persimmons

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2023/v45i102201

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/105181

Original Research Article

Received: 20/06/2023
Accepted: 24/08/2023
Published: 02/09/2023

ABSTRACT

Because of fruit fly incidence, importing countries impose phytosanitary barriers to prevent the entry and spread of infested fruits. When fruits are not grown in fruit fly-free areas, some countries require quarantine treatments for fruit disinfestation before or during shipping. The objective of this study was to evaluate the use of a hydrothermal treatment as a quarantine treatment for 'Fuyu' persimmons infested with two fruit fly species to maintain fruit quality. Hot water treatment (HWT) was applied to eggs and third-instar larvae of *Ceratitis capitata* and *Anastrepha fraterculus in vitro*

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at 42º, 44º, 46º, 48º, and 50 ºC for 20, 30, 40, 50, 60, and 90 min. Persimmon fruit infested by *C. capitata* and *A. fraterculus* eggs and third-instar larvae were subjected to 44 ºC for 60 and 90 min, 46 °C for 20 and 30 min; and 48 °C for 20 min. Untreated infested fruits were used to estimate the rate of fruit fly infestation. Pulp firmness, skin and pulp colors, titratable acidity, pH, and total soluble solids were measured to evaluate the effect of HWT on fruit quality. The increase in temperature and immersion time decreased the pupation and emergence of adults of *C. capitata* and *A. fraterculus in vitro*. *C. capitata* eggs treated *in vitro* at temperatures ≥ 46 ºC for 30 min did not produce pupae. *A. fraterculus* eggs treated *in vitro* at 46 ºC for 20 min exhibited no larval hatching. No pupae were obtained from third-instar larvae of both fruit fly species treated at 44 ºC for 60 min. Infested 'Fuyu' persimmons treated at 44 ºC for 90 min did not exhibit the emergence of adults of *C. capitata* or *A. fraterculus*. Except for pH, in general, 'Fuyu' persimmons subjected to HWT demonstrated no alterations of skin or pulp texture, skin coloration, titratable total acidity, or total soluble solids. The results suggest that HWT is a potential quarantine treatment for the export of 'Fuyu' persimmons.

Keywords: Ebenaceae; post-harvest; heat treatment; Ceratitis capitata; Anastrepha fraterculus.

1. INTRODUCTION

Brazil is the fourth largest producer of persimmon in the world after China, Japan, and South Korea [1]. In 2021, the state of São Paulo was the main Brazilian producer of persimmon with 78,609 tons harvested from 3,167 hectares [2].

Persimmon (*Diospyros kaki* L.) (Ebenaceae) is commonly infested by two fruit flies (Tephritidae) in Brazil: the native *Anastrepha fraterculus* (Wied.) and the exotic *Ceratitis capitata* (Wied.) [3, 4]. In addition to the direct economic damage, quarantine restrictions limit the export of Brazilian persimmons because the main growing areas are infested by both fruit fly species. Egg and larval durations at 25 ºC range, respectively, from 2.4–2.6 days to 7.9–9.5 days for *C. capitata* [5] and from 2.6–3.2 days to 11.0–14.0 days for *A. fraterculus* [6].

Regulatory quarantine actions prevent the transportation of infested commodities and reduce the risk of introducing pests into pest-free areas [7]. Consequently, the risk of commercialization of asymptomatic fruits [8] from infested growing areas requires post-harvest treatments to ensure the safety of the product that is exported and to comply with the quarantine regulations of the importing countries. The detection of even a single quarantine pest in fresh fruit may result in the immediate destruction of the entire load at the expense of the exporter or may even provoke the ban of future shipments to the market destination [9]. Pestiferous fruit flies lay their eggs under the skins of fruits, and larva feed on pulp until they leave for pupation in the soil. Consequently, both egg and larval stages may occur during fruit harvesting and commercialization. Many factors hinder fruit inspection, including the age of the Tephritidae immatures, fruit size and ripeness, and the ability of inspectors [10].

Physical treatments include the application of heat in the form of hot water, hot air, or vapor, which are used to increase the temperature of the host commodity above the thermal limits of survival of the pest [11, 12]. Hot water treatment (HWT) was initially used to kill pathogens (fungi and bacteria) and was effectively tested against fruit flies (Tephritidae) in bananas, papaya, and mango during the 1980s [13].

The efficacy of HWT depends on the time of exposure needed to reach the desired temperature in the center of the fruit [14]. The lethal dose varies according to the tephritid species. Instead of killing the eggs or larvae in the fruit, the quarantine treatment should provide quarantine security by preventing the emergence of adults [15, 16, 17].

The temperature vs. time of exposure during heat treatment is determined by the thermal tolerance of the different stages of the target pest in the commodity, followed by the probit analysis for estimation of the time required to kill the most heat-tolerant stage of the pest [9, 18]. The third instar is considered the most heat-tolerant larval stage of tephritids [18, 19, 20].

'Keitt', 'Haden', and 'Tommy Atkins' mangoes are disinfested from *Anastrepha ludens* (Loew) and *A. obliqua* (Macquart) by immersion in water at 46.l °C for 90 min [21]. The export of Brazilian mangos to the USA began in 1992, using HWT to disinfest fruit flies. However, many

commodities do not tolerate immersion in hot water [22]. If a certain fruit cannot tolerate high temperatures for short periods, it may tolerate lower temperatures for longer treatment times [23]. Therefore, this study aimed to prevent the pupation and emergence of *A. fraterculus* and *C. capitata* in non-astringent 'Fuyu' persimmons, using HWT by (i) comparing the tolerance of eggs and larvae of both species to the temperature vs. time of exposure *in vitro* and in persimmons, (ii) estimating the probit values for the HWT times required for eggs and larvae, and (iii) evaluating the physicochemical parameters of persimmons subjected to HWT.

2. MATERIALS AND METHODS

2.1 Experimental Sites

The tests with insects and HWT were conducted at the Laboratory of Economic Entomology (LEE) of the Biological Institute. Physicochemical analyses were performed at the Department of Food Technology, School of Food Engineering, State *University* of Campinas, both located in Campinas (SP), Brazil.

2.2 Insects and Fruits

The eggs and larvae of *C. capitata* (medfly) and *A. fraterculus* used in the experiments were obtained from the colonies maintained at LEE since 1993 and 2002, respectively. Medfly larvae were reared on an artificial diet, whereas *A. fraterculus* larvae were reared on papaya fruit [24]. The mature 'Fuyu' persimmons used here were obtained from the Food Supply Center (CEASA) in Campinas without physical damage, pest, or disease symptoms. For the analysis of egg infestation, the average weight of the fruit was 176.15 g (120–222 g) for *C. capitata* and 175.35 g (122–222 g) for *A. fraterculus*. For the analysis of larval infestation, the average weight of the fruit was 177.11 g (138–222 g) for *C. capitata* and 174.06 g (124–216 g) for *A. fraterculus*. The average weight of persimmons for the physicochemical tests was 157.25 g (128–180 g).

2.3 Heating Chamber

The equipment used for HWT was a Dubnoff model 304-TPA water bath, manufactured by Ethik Technology. The equipment had a capacity of 36 L, with thermostat-controlled heating, and constant agitation at 10 rpm.

2.4 Insect *In vitro* Tests

2.4.1 Hydrothermal treatment against *C. capitata* and *A. fraterculus* eggs

Medfly eggs were collected from voile cloth (which serves as an oviposition surface) located at the wide sides of the rearing cages. The egg-collecting apparatus used for *A. fraterculus* was described by Baldo et al. [16].

Eggs of *C. capitata* and *A. fraterculus* with a maximum of 24 h of age were submitted to hydrothermal treatment in a water bath at temperatures of 41, 42, 44, 46, 48, and 50 ± 0.5 ºC combined with immersion times of 7, 15, 20, 30, 40, 50, 60, and 90 min. We used an untreated control (without heat application) for each tested temperature. For the control, eggs were kept at 25ºC in crucibles lined with moistened filter paper until transferred to an artificial diet. Each treatment (temperature × time) involved eight repetitions with 20 eggs of *C. capitata* and *A. fraterculus* per repetition. Eggs subjected to hydrothermal treatment were counted under a stereoscopic microscope and transferred to porcelain crucibles with a capacity of 50 mL, which contained 10 mL of distilled water. The temperature during the treatment was constantly measured by a mercury column thermometer.

After the application of each treatment, *C. capitata* eggs were transferred to plastic cups containing 50 g of an artificial diet, closed with voile cloth, and bound with an elastic band. After 10 to 15 days of treatment, the number of pupae in each replication was counted. Then, medfly pupae were transferred to 10-cm-diameter Petri dishes. Approximately 15–20 days later, the adults were counted. In the case of *A. fraterculus*, the treated eggs were just transferred to small Petri dishes containing a round piece of filter paper moistened at the bottom, enough so that the eggs did not dehydrate. After 5 days, the number of hatched larvae in each replicate was counted. All materials were stored in a BOD chamber at 25 °C.

2.4.2 Hydrothermal treatment against *C. capitata* and *A. fraterculus* larvae

Third-instar larvae of *C. capitata* and *A. fraterculus* were submitted to hydrothermal treatment in a water bath at temperatures of 41, 42, 44, 46, 48, and 50 ± 0.5 ºC with immersion times of 7, 15, 20, 30, 40, 50, 60, and 90 min.

We added a control (without immersion in the water bath) for each temperature. Each treatment (temperature × time) involved eight replications with 20 larvae per replication. The larvae were transferred to porcelain crucibles with a capacity of 50 mL, which contained containing 10 mL of distilled water, and were subjected to HWT. The immersion time was counted starting from the moment when the last crucible was placed in the preheated water bath at the desired temperature for each treatment. After the application of each treatment, *C. capitata* larvae were placed in plastic pots containing an artificial diet (described above) and *A. fraterculus* larvae were transferred to plastic pots containing vermiculite. Both pots were closed with voile cloth and bound with an elastic band. After 10–12 days, the number of pupae in each repetition was counted and the pupae were transferred to 10-cm-diameter Petri dishes. Approximately 10–12 days later, adults were counted. All materials were stored in the BOD chamber at 25 °C.

2.5 Hydrothermal Treatment of Infested Persimmon Against *C. capitata* and *A. fraterculus* Eggs and Larvae

The persimmons were washed under running water, dried with a paper towel, and placed into acrylic cages made with nylon screen (40 × 40 × 50 cm) and containing *C. capitata* or *A. fraterculus* adults. The fruit were subjected to natural infestation for approximately 24 h, using 10 sexually mature females per fruit. After infestation, the persimmons were immediately submitted to HWT.

In the case of larvae, persimmons were stored for 8 days when infested with *C. capitata* and 10 days when infested with *A. fraterculus.* These periods were necessary for larval development (to third-instar larvae). The chosen treatments (temperature × time employed) were based on the results obtained by the *in vitro* treatments. Combined treatments of 44 and 46 ± 0.5 ºC for 60 and 90 min, as well as 48 ± 0.5 ºC for 20 min, were tested against eggs and larvae in fruit. Infested persimmons without exposure to HWT were used as the control under the same conditions as the treated fruit.

We used 10 repetitions, where each persimmon was considered one repetition. After applying the treatment, the persimmons were stored in plastic pots containing vermiculite, covered with a white voile cloth, and stored at 25ºC and 70% relative humidity (RH). After 14 to 20 days after infestation with *C. capitata* and *A. fraterculus,* respectively, the fruit were removed and pupae were counted. After approximately 15 days, the adults were counted.

2.6 Physicochemical Assays

'Fuyu' persimmons three to four days after harvest were used in the physicochemical tests. Six groups (treatments) were evaluated: a control (without HWT), groups treated by immersion at 44 ± 0.5 ºC for 60 and 90 min, groups treated by immersion at 46 ± 0.5 ºC for 20 and 30 min, and a group treated by immersion at 48 ± 0.5 ºC for 20 min. Each treatment comprised three repetitions, considering each fruit one repetition. The physicochemical analyses were carried out at the Laboratory of Instrumental Analysis of the Faculty of Food Engineering (FEA) of the State University of Campinas (UNICAMP). Fruit were submitted to the evaluation of skin and pulp texture, skin coloration, titratable total acidity (TA), pH, and total soluble solids (TSS). The methodology for the analysis was described by Baldo et al. [16]. Maximum forces at the point of penetration on skin and pulp (N) were used as the firmness value, as well as the penetration distance reached at the maximum force (mm).

2.7 Statistical Analysis

For the *in vitro* hydrothermal treatments, the data were analyzed using the Assistat 7.7 software [25]. The means of the treatments *in vitro*, those of infested persimmons, and the physicochemical parameters were compared using Tukey's test at 5% probability, under a completely randomized design. For the *in vitro* conditions, the factorial design was 6 (temperature) × 9 (times) and data were submitted to Probit analysis using the StatPlus software [26] to estimate the lethal times (LT_{50}, Lt_{90}, and LT_{99}) for achieving the desired criterium (no adult emergence).

3. RESULTS

3.1 HWT against *C. capitata* Immatures *in Vitro*

3.1.1 Eggs

The numbers of pupae and adults were significantly different among tested temperatures and immersion times. Interactions were found between exposure times and temperatures, as measured by the number of pupae (F = 21.50; *df*

= 5, 40; p < 0.05) and adults (F = 11.38; *df* = 5, 40; p < 0.05) that developed from treated medfly eggs. When *C. capitata* eggs were treated at temperatures ≥ 46 ºC for 30 min, a significantly lower mean number of pupae and adults developed compared to temperatures ≤ 42ºC for the same or longer immersion times (Table 1).

Regardless of immersion time, *C. capitata* eggs treated at 41ºC showed similar pupation and emergence to the control. At 42ºC and 44ºC, the minimum immersion times of 15 min and 7 min significantly reduced pupation and emergence, respectively. Eggs treated at 42ºC and 44ºC did not completely avoid pupation and emergence, regardless of immersion time (Table 1). Eggs treated for 30, 20, and 7 min at 46, 48, and 50 ºC, respectively, prevented the pupation and emergence of *C. capitata*.

Treating eggs *in vitro* under the highest temperatures (≥ 46 ºC) for shorter treatment times (7–30 min) prevented the pupation and emergence of *C. capitata*. The inverse may also be true because, for the lowest temperatures (≤ 44 ºC), the longest exposure times were necessary to reduce pupation and adult emergence (≥ 60 min). The lethal times (LT_{99}) for treated eggs at 46 ºC and 48º C were estimated at 35.59 and 23.04 min, respectively (Table 2) (i.e., increasing the temperature from 46 ºC to 48 ºC decreased the LT_{99} by 35.3% to prevent adult emergence).

3.1.2 Larvae

Interactions between temperature levels and immersion times were found and were measured by the number of pupae (F = 57.52; *df* = 5, 40; p < 0.05) and adults (F = 31.84; *df* = 5, 40; p < 0.05) that emerged after third-instar larvae were treated *in vitro*. Temperatures above 46 ºC significantly decreased the number of surviving individuals (pupae and adults) from third-instar larvae compared to lower temperatures (≤ 44 ºC for up to 50 min). Temperatures ≤ 44 ºC for up to 15 min did not result in reduced population compared to the control. However, emergence was reduced when third-instar larvae were treated at 44 ºC for all immersion times (Table 3). At lower tested temperatures (≤ 44 ºC), the immersion time had to be longer (60–90 min) to prevent insect development.

Regardless of immersion time, no adult emerged from third-instar larvae treated at ≥ 48 ºC for 15 min or longer (Table 3). Although pupae were obtained in groups treated for 20 and 15 min at 46 ºC and 48 ºC, respectively, the treated larvae failed to reach the adult stage. The lethal doses (LT_{99}) to prevent adult emergence from third-instar *C. capitata* larvae subjected to HWT were estimated at 24.23 min at 46 ºC and 17.29 min at 48 ºC (Table 2).

3.2 Hydrothermal Treatment against Immatures of *A. fraterculus in Vitro*

3.2.1 Eggs

Interactions were found between temperatures and immersion times, as measured by the number of hatched larvae (F = 6.05; *df* = 5, 40; p < 0.05) from treated eggs of *A. fraterculus*. Eggs treated for 20 min at 46ºC, 15 min at 48 ºC, and 7 min at 50 ºC prevented *A. fraterculus* larval hatching (Table 4). Eggs immersed for 90 min did not show larval hatching for all HWT temperatures. Neither did eggs subjected to 50ºC for all immersion times. Even eggs treated at 41 ºC and 42 ºC exhibited a significant reduction in larval hatching compared to the untreated control. The egg mortality increased substantially with increasing temperature and immersion times. The lethal times (LT_{99}) necessary to cause 99% lethality of *A. fraterculus* eggs were estimated at 20.81 and 13.55 min at temperatures of 46ºC and 48ºC, respectively (Table 5).

3.2.2 Larvae

Interactions were found between temperature and immersion time, as measured by the number of pupae (F = 58.82; *df* = 5, 40; p < 0.05) and adults (F = 45.36; *df* = 5, 40; p < 0.05). Temperatures ≤ 42ºC did not completely prevent the pupation and emergence from treated third-instar larvae of *A. fraterculus,* although larvae treated for 15 min at 42ºC exhibited a slight reduction in those parameters (Table 6). Increasing the temperature from 42ºC to 44ºC resulted in the highest reduction in the number of pupae and adults compared to the other temperatures.

Immersion times of 90 min at 44ºC, 40 min at 46ºC, 20 min at 48ºC, and 15 min at 50ºC prevented the pupation of *A. fraterculus.* The estimated lethal times (LT_{99}) to prevent adult emergence from third-instar larvae were 26.62 min at 46 ºC and 18.38 min at 48 ºC (Table 5).

Table 1. Pupae and adult of *C. capitata* obtained after egg (n=20) exposure to HWT at different temperatures and times *in vitro* conditions

Temp.	Time (minutes)								
	0	7	15	20	30	40	50	60	90
Avg no. Pupae ± SE									
41º C	18.87 ± 0.40 aA	17.50 ± 0.65 aA	17.50 ± 0.82 aA	17.25 ± 0.37 aA	18.00 ± 0.38 aA	17.75 ± 0.62aA	17.00 ± 0.46aA	18.37 ± 0.38aA	17.00 ± 0.57aA
42 ºC	18.25 ± 0.59abA	15.12 ± 0.93abAB	13.62 ± 1.03bBC	12.50 ± 1.39bBCD	10.50 ± 1.35bCD	9.87 ± 0.88 bD	6.00 ± 0.57 bE	6.62 ± 0.56 bE	5.87 ± 0.69 bE
44 ºC	14.62 ± 0.73 cA	14.37 ± 0.38 bA	13.75 ± 0.84 bA	5.37 ± 0.68 cB	4.37 ± 1.21 cBC	2.37±1.03cBCD	2.00 ±0.68cCD	1.37 ±0.53cCD	0.37 ± 0.26 cD
46 ºC	18.00 ± 0.76abA	16.50 ± 0.53 abA	13.00 ± 0.94 bB	0.25 ± 0.25 dC	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 cC
48 ºC	15.37 ± 0.71bcA	6.50 ± 2.60 cB	1.87 ± 0.93 cC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 cC
50 ºC	16.00±1.56abcA	0.00 ± 0.00 dB	0.00 ± 0.00 cB	0.00 ± 0.00 dB	0.00 ± 0.00 dB	0.00 ± 0.00 cB	0.00 ± 0.00 cB	0.00 ± 0.00 cB	0.00 ± 0.00 cB
Avg no. Adults ± SE									
41º C	18.12 ± 0.67 aA	17.12 ± 0.72 aA	17.37 ± 0.86aA	16.37 ± 0.68 aA	17.37 ± 0.46 aA	17.50 ± 0.57 aA	16.75 ± 0.45aA	16.62 ± 1.02aA	16.75 ± 0.59aA
42 ºC	18.00 ± 0.53 aA	13.75 ± 0.92 bB	12.25 ± 0.84bBC	12.12 ± 1.33 bBC	10.12 ± 1.47 bC	9.75 ± 0.92bCD	5.87 ± 0.64 bE	6.62 ±0.53bDE	5.75 ± 0.65 bE
44 ºC	14.25 ± 0.59 bA	8.62 ± 0.98 cB	6.87 ± 0.99 cBC	5.00 ± 0.68 cCD	3.87 ± 0.99cCDE	1.87±0.88cDEF	1.87±0.69cDEF	1.25 ± 0.53cEF	0.37 ± 0.26 cF
46 ºC	17.62 ± 0.71 aA	8.12 ± 0.44 cB	6.25 ± 0.80 cB	0.12 ± 0.13 dC	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 cC
48 ºC	15.25 ± 0.67abA	5.75 ± 2.48 cB	1.75 ± 0.86 dC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 cC
50 ºC	15.50 ± 1.56abA	0.00 ± 0.00 dB	0.00 ± 0.00 dB	0.00 ± 0.00 dB	0.00 ± 0.00 dB	0.00 ± 0.00 cB	0.00 ± 0.00 cB	0.00 ± 0.00 cB	0.00 ± 0.00 cB

Values followed by different lowercase letters within a column and by different letters within a row are significantly different ($p < 0.05$) based on Tukey's multiple range test

Table 2. Probit model estimates of time required to achieve 50%, 90%, and 99% mortality of different immature stages of *C. capitata* after HWT *in vitro* conditions

Temp.	Lethal time (minutes)		
	LT_{50}	LT_{90}	LT_{99}
	Eggs		
46 ºC	6.86	16.99	35.59
48 ºC	5.02	11.62	23.04
	Larvae		
46 ºC	7.14	13.99	24.23
48 ºC	5.75	10.54	17.29

Table 3. Pupae and adult of *C. capitata* obtained after larvae (n=20) exposure to HWT at different temperatures and times *in vitro* conditions

Temp.	Time (minutes)								
	0	7	15	20	30	40	50	60	90
	Avg no. Pupae ± SE								
41º C	19.75 ± 0.25 aA	19.00 ± 0.38 aA	19.25 ± 0.37 aA	19.37 ± 0.26 aA	19.12 ± 0.30 aA	19.25 ± 0.25 aA	18.87 ± 0.35 aA	18.37 ± 0.92 aA	17.62 ± 1.05 aA
42 ºC	20.00 ± 0.00 aA	18.87 ± 0.48 aA	18.37 ± 0.56 aA	15.12 ± 0.68 bB	9.50 ± 0.82 bC	8.75 ± 0.25 bC	8.87 ± 0.68 bC	8.25 ± 0.71 bC	0.50 ± 0.27 bE
44 ºC	19.75 ± 0.16 aA	18.50 ± 0.42 aA	18.37 ± 0.42 aA	11.75 ± 1.36 cB	6.75 ± 1.35 cC	5.25 ± 1.00 bC	2.87 ± 0.40 cD	1.00 ± 0.33 cDE	0.00 ± 0.00 bE
46 ºC	19.62 ± 0.18aA	16.25 ± 0.75 bB	4.12 ± 1.06 bC	0.62 ± 0.26 dD	0.00 ± 0.00 dD	0.00 ± 0.00 cD	0.00 ± 0.00 dD	0.00 ± 0.00 cD	0.00 ± 0.00 bD
48 ºC	19.25 ± 0.31aA	12.37 ± 0.89 cB	1.75 ± 0.41 cC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 bC
50 ºC	19.87 ± 0.13aA	2.12 ± 0.79 dB	0.00 ± 0.00 cB	0.00 ± 0.00 dB	0.00 ± 0.00 dB	0.00 ± 0.00 cB	0.00 ± 0.00 dB	0.00 ± 0.00 cB	0.00 ± 0.00 bB
	Avg no. Adults ± SE								
41º C	19.12 ± 0.30 aA	16.00 ± 0.71 aB	14.37 ± 0.53 aB	15.00 ± 0.60 aB	16.12 ± 0.44 aB	16.50 ± 0.76 aB	16.00 ± 0.76 aB	15.37 ± 1.08 aB	10.37 ± 0.96 aC
42 ºC	19.75 ± 0.16 aA	16.00 ± 0.68 aB	15.12 ± 1.09 aB	13.75 ± 0.65 aB	7.87 ± 0.74 bC	4.00 ± 0.33 bD	6.37 ± 0.86 bCD	6.62 ± 0.75 bC	0.12 ± 0.13 bE
44 ºC	19.12 ± 0.40 aA	11.12 ± 0.88 bB	10.62 ± 0.53 bB	9.50 ± 1.22 bB	5.37 ± 1.07 cC	2.87 ± 0.67 bD	1.50 ± 0.27 cDE	0.12 ± 0.13 cE	0.00 ± 0.00 bE
46 ºC	19.00 ± 0.33 aA	9.87 ± 0.58 bB	2.62 ± 1.02 cC	0.00 ± 0.00 cD	0.00 ± 0.00 dD	0.00 ± 0.00 cD	0.00 ± 0.00 cD	0.00 ± 0.00 cD	0.00 ± 0.00 bD
48 ºC	17.75 ± 0.37 aA	7.00 ± 0.42 cB	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 bC
50 ºC	19.37 ± 0.32 aA	2.12 ± 0.79 dB	0.00 ± 0.00 dB	0.00 ± 0.00 cB	0.00 ± 0.00 dB	0.00 ± 0.00 cB	0.00 ± 0.00 cB	0.00 ± 0.00 cB	0.00 ± 0.00 bB

Values followed by different lowercase letters within a column and by different uppercase letters within a row are significantly different ($p < 0.05$) based on Tukey's multiple range test

Table 4. Hatched larvae of *A. fraterculus* obtained after exposure eggs (n=20) to HWT at different temperatures and times *in vitro* conditions.

Temp.	Time (minutes)								
	0	7	15	20	30	40	50	60	90
	Avg no. Larvae ± SE								
41 ºC	12.00 ± 0.85aA	7.75 ± 1.31aB	7.00 ± 1.10aB	6.37 ± 0.96aB	3.12 ± 0.91aC	2.00 ± 0.42aCD	1.75 ± 0.53aCD	1.12 ± 0.35aCD	0.00 ± 0.00aD
42 ºC	10.12 ± 0.52abA	6.00 ± 1.07abB	4.87 ± 0.55 bBC	4.00 ± 0.60bBCD	3.00 ± 0.53aCDE	1.87 ± 0.44abDEF	1.37 ± 0.18 aEF	1.00 ± 0.27aEF	0.00 ± 0.00aF
44 ºC	10.00 ± 0.60 bA	5.25 ± 1.19 bB	3.00 ± 0.46 bcC	1.62 ± 0.50 cCD	1.62 ± 0.50abCD	1.12 ± 0.40abCD	0.50 ± 0.19aD	0.37 ± 0.26aD	0.00 ± 0.00aD
46 ºC	10.50 ± 0.57abA	2.37 ± 0.56 cB	1.12 ± 0.40cdBC	0.00 ± 0.00 cC	0.00 ± 0.00 bC	0.00 ± 0.00 bC	0.00 ± 0.00 bC	0.00 ± 0.00 bC	0.00 ± 0.00aC
48 ºC	10.00 ± 0.38 bA	1.50 ± 0.33cdB	0.00 ± 0.00 dB	0.00 ± 0.00 cB	0.00 ± 0.00 bB	0.00 ± 0.00 bB	0.00 ± 0.00 bB	0.00 ± 0.00aB	0.00 ± 0.00 aB
50 ºC	11.12 ± 0.79abA	0.00 ± 0.00 dB	0.00 ± 0.00 dB	0.00 ± 0.00 cB	0.00 ± 0.00 bB	0.00 ± 0.00 bB	0.00 ± 0.00 bB	0.00 ± 0.00 aB	0.00 ± 0.00aB

Values followed by different lowercase letters within a column and by different uppercase letters within a row are significantly different ($p < 0.05$) based on Tukey's multiple range test

Table 5. Probit model estimates of time required to achieve 50%, 90%, and 99% mortality of different immature stages of *A. fraterculus* after HWT *in vitro* conditions

Temp.	Lethal time (minutes)		
	LT_{50}	LT_{90}	LT_{99}
		Eggs*	
46 ºC	2.60	8.19	20.81
48 ºC	2.60	6.46	13.55
	Larvae**		
46 ºC	6.82	14.44	26.62
48 ºC	4.71	9.98	18.38

**Lethal time for larval hatching **Lethal time for no adult emergence*

Table 6. Pupae and adults of *A. fraterculus* obtained after exposure of third-instar larvae to HWT at different temperatures and times *in vitro* conditions

Temperature	Time of exposure (minutes)								
	0	7	15	20	30	40	50	60	90
	Avg no. Pupae ± SE								
41 ºC	18.50 ± 0.53 aA	18.00 ± 0.60 aA	18.00 ± 0.53 aA	18.12 ± 0.58 aA	18.00 ± 0.53 aA	17.25 ± 0.77 aA	18.12 ± 0.44 aA	16.75 ± 0.75 aA	18.00 ± 0.65 aA
42 ºC	19.25 ± 0.31 aA	18.62 ± 0.38 aA	15.87 ± 0.64 bB	14.50 ± 0.63 bB	12.50 ± 0.57 bC	12.12 ± 0.64 bC	10.00 ± 0.71 bD	8.87 ± 0.44 bD	6.25 ± 0.59 bE
44 ºC	19.37 ± 0.26 aA	14.87 ± 0.61 bB	14.37 ± 0.62 bB	7.37 ± 0.60 cC	7.00 ± 0.60 cD	6.25 ± 0.59 cC	4.25 ± 0.53 cD	1.00 ± 0.33 cE	0.00 ± 0.00 cE
46 ºC	19.00 ± 0.38 aA	15.25 ± 0.65 bB	4.00 ± 0.46 cC	1.37 ± 0.38 dD	0.50 ± 0.27 dD	0.00 ± 0.00 dD	0.00 ± 0.00 dD	0.00 ± 0.00 cD	0.00 ± 0.00 cD
48 ºC	19.25 ± 0.25 aA	11.62 ± 0.60 cB	1.37 ± 0.38 dC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 cC
50 ºC	19.37 ± 0.26 aA	4. 75 ± 0.37 dB	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 cC
	Avg no. Adults ± SE								
41º C	17.37 ± 0.68 aA	16.50 ± 0.68 aA	17.12 ± 0.61 aA	16.87 ± 0.55 aA	16.75 ± 0.80 aA	16.25 ± 0.80 aA	16.25 ± 0.75 aA	15.37 ± 0.65 aA	16.50 ± 0.65 aA
42 ºC	17.87 ± 0.55 aA	17.50 ± 0.60 aA	14.25 ± 0.45 bB	13.25 ± 0.75 bB	11.12 ± 0.58 bC	11.00 ± 0.76 bCD	9.00 ± 0.80 bDE	7.12 ± 0.64 bE	5.00 ± 0.46 bF
44 ºC	18.50 ± 0.38 aA	13.25 ± 0.53 bB	13.50 ± 0.94 bB	4.75 ± 0.62 cD	3.50 ± 0.46 cD	3.12 ± 0.52 cCD	1.62 ± 0.32 cDE	0.75 ± 0.25 cE	0.00 ± 0.00 cE
46 ºC	18.37 ± 0.42 aA	9.62 ± 0.86 cB	1.75 ± 0.25 cC	0.75 ± 0.31 dC	0.12 ± 0.13 dC	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 cC
48 ºC	18.12 ± 0.35 aA	4.87 ± 0.44 dB	0.75 ± 0.25 cC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 cC
50 ºC	17.87 ± 0.40 aA	2.12 ± 0.35 eB	0.00 ± 0.00 cC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 dC	0.00 ± 0.00 cC	0.00 ± 0.00 cC	0.00 ± 0.00 cC

Values followed by different lowercase letters within a column and by different uppercase letters within a row are significantly different (p < 0.05) based on Tukey's multiple range test

Table 7. Pupae and adults per fruit after HWT of 'Fuyu' persimmons infested with immature stages of *C. capitata* and *A. fraterculus*

Treatment	Treated eggs		Treated 3rd Instar Larvae	
temp. vs minutes	Pupae	Adults	Pupae	Adults
	C. capitata			
Control	20.0 ± 2.50a	15.70 ± 2.09a	25.70 ± 7.88a	17.90 ± 5.18a
44º C 60'	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b
44º C 90'	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b
46º C 20'	8.80 ± 2.71b	6.80 ± 1.78b	0.20 ± 0.20b	0.10 ± 0.10b
46º C 30'	0.00 ± 0.00c	0.00 ± 0.00c	1.10± 0.77b	0.60 ± 0.40b
48º C 20'	0.00 ± 0.00c	0.00 ± 0.00c	0.30 ± 0.30b	0.20 ± 0.20b
	A. fraterculus			
Control	16.30 ± 1.96a	12.40 ± 1.75a	13.30 ± 5.29a	6.00 ± 2.66a
44º C 60'	0.00 ± 0.00b	0.00 ± 0.00b	0.70 ± 0.60c	0.30 ± 0.21c
44º C 90'	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00c
46º C 20'	0.00 ± 0.00b	0.00 ± 0.00b	7.20 ± 1.99b	3.70 ± 1.43b
46º C 30'	0.00 ± 0.00b	0.00 ± 0.00b	7.60 ± 4.16b	4.30 ± 1.93b
48º C 20'	0.00 ± 0.00b	0.00 ± 0.00b	9.30 ± 3.76b	3.70 ± 1.33b

Values followed by different letters within a column by transect are significantly different (p < 0.05) based on Tukey's multiple range test

Table 8. Mean values of firmness in 'Fuyu' persimmons subjected to HWT and stored at 25 ± 1.0 ºC for 1 and 7 days

Temp. vs time	Storage Period (days)					
	1	7	1	7	1	7
	Maximum force on skin (N)		penetration distance at the maximum force (mm)		Maximum forces on pulp (N)	
Control	9.23 ± 0.31^{ns}	5.03 ± 1.50^{ns}	1.90 ± 0.08c	3.29 ± 1.03^{ns}	2.09 ± 0.26^{ns}	0.96 ± 0.49^{ns}
44º C 60'	7.96 ± 0.31	6.27 ± 0.41	2.47 ± 0.18ab	5.99 ± 1.25	1.69 ± 0.19	0.66 ± 0.07
44º C 90'	8.93 ± 0.34	5.85 ± 0.12	2.65 ± 0.11a	7.26 ± 0.6	1.96 ± 0.25	0.59 ± 0.20
46º C 20'	7.90 ± 0.33	6.62 ± 0.66	2.06 ± 0.09bc	5.87 ± 1.11	1.87 ± 0.41	0.83 ± 0.28
46º C 30'	9.03 ± 0.32	6.24 ± 0.30	2.22 ± 0.01abc	5.39 ± 1.04	1.77 ± 0.30	0.76 ± 0.25
48º C 20'	7.55 ± 1.17	6.92 ± 0.63	2.03 ± 0.12bc	3.30 ± 0.40	1.64 ± 0.55	1.06 ± 0.41

Values followed by different letters within a column are significantly different (p < 0.05) based on Tukey's multiple range test

Table 9. Mean values of skin color for 'Fuyu' persimmons subjected to HWT and stored at 25 ± 1.0 ºC for 1 and 7 days

Temp. vs time	Storage Period (days)					
	1	7	1	7	1	7
	(L)		Chroma (a)		Hue (b)	
Control	53.92 ± 1.30^{ns}	42.94 ± 3.00^{ns}	42.34 ± 1.27^{ns}	34.74 ± 3.92^{ns}	89.32 ± 4.32^{ns}	39.10 ± 3.21^{ns}
44º C 60'	53.44 ± 1.90	43.90 ± 2.87	39.30 ± 2.16	29.01 ± 2.59	91.08 ± 2.69	40.82 ± 4.39
44º C 90'	52.84 ± 0.80	40.81 ± 0.89	40.08 ± 2.28	24.92 ± 2.59	88.89 ± 1.05	34.85 ± 2.03
46º C 20'	51.22 ± 1.69	43.04 ± 2.11	44.02 ± 1.20	23.49 ± 1.57	87.79 ± 2.78	37.33 ± 3.82
46º C 30'	53.38 ± 1.12	46.34 ± 2.40	41.53 ± 2.99	29.41 ± 1.64	90.00 ± 1.48	42.45 ± 3.29
48º C 20'	52.03 ± 0.19	44.17 ± 0.79	39.98 ± 1.12	30.82 ± 2.02	83.69 ± 4.28	36.91 ± 0.62

Values followed by different letters within a column are significantly different (p < 0.05) based on Tukey's multiple range test

Table 10. Mean values of acidity, pH and soluble solids in persimmons submitted to HWT and stored at 25 ± 1.0 ºC for 1 and 7 days

Treatment (temperature/time)	Storage Period (days)					
	1	7	1	7	1	7
	titratable total acidity (%)		pH		Soluble solids (ºBrix)	
25º C 0'	44.11 ± 0.04^{ns}	37.50 ± 0.02^{ns}	5.95 ± 0.07bB	6.30 ± 0.17aA	15.70 ± 0.29^{ns}	15.30 ± 0.2^{ns}
44º C 60'	42.08 ± 0.08	38.81 ± 0.03	6.24 ± 0.04abA	6.19 ± 0.03aA	14.79 ± 0.55	14.37 ± 0.38
44º C 90'	37.60 ± 0.04	39.95 ± 0.04	6.34 ± 0.02aA	6.05 ± 0.09abB	14.97 ± 0.24	14.97 ± 0.24
46º C 20'	48.64 ± 0.08	38.81 ± 0.04	6.24 ± 0.02abA	5.88 ± 0.03bB	15.53 ± 0.52	14.33 ± 0.55
46º C 30'	50.00 ± 0.06	39.48 ± 0.04	6.33 ± 0.10aA	5.92 ± 0.03bB	15.97 ± 0.99	14.37 ± 0.37
48º C 20'	43.34 ± 0.02	49.91 ± 0.02	6.30 ± 0.09aA	5.88 ± 0.06bB	15.17 ± 0.74	14.20 ± 0.44

Values followed by different letters within a column are significantly different (p < 0.05) based on Tukey's multiple range test

3.3 Infested Persimmons Subjected to HWT

3.3.1 Eggs and third-instar larvae of *C. capitata*

All HWT treatments prevented pupation, except for the exposure of *C. capitata* eggs to 46 ºC for 20 min (Table 7). When this temperature was applied for 20 or 30 min, pupation and emergence from medfly third-instar larvae were not prevented. 'Fuyu' persimmons infested with third-instar larvae and treated at 44ºC for 60 or 90 min prevented the full pupal stage. Fruit infested with third-instar larvae and treated at 46ºC for 20 or 30 min or 48ºC for 20 min exhibited surviving pupae and adults, although in small numbers.

3.3.2 Eggs and third-instar larvae of *A. fraterculus*

All HWT treatments were effective in preventing the pupation and emergence of *A. fraterculus* adults from eggs (Table 7). In the experiments carried out with third-instar larvae, only HWT at 44 ºC for 90 min prevented pupation. Because of the shorter immersion times, fruit treated at 46 ºC and 48 ºC did not prevent the pupation and emergence of *A. fraterculus* and were less effective than persimmons treated at 44 ºC for 60 min.

3.4 Physicochemical Analysis of 'Fuyu' Persimmons Subjected to HWT

There were no differences regarding skin firmness among persimmons subjected to HWT and stored for 1 day ($F = 1.60$; $df = 5, 12$; $p > 0.05$) and 7 days ($F = 0.78$; $df = 5, 12$; $p > 0.05$) (Table 8). Moreover, there was no interaction between HWT and storage period for skin firmness ($F = 1.98$; $df = 5, 24$; $p > 0.05$). Nonetheless, regardless of HWT, there were differences between the two storage periods with a significant reduction in skin firmness ($F = 35.69$; $df = 1, 24$; $p < 0.05$) for persimmons stored for 7 days. The same effects occurred for pulp firmness of fruit stored for 1 day ($F = 0.24$; $df = 5, 12$; $p > 0.05$) and 7 days ($F = 0.32$; $df = 5, 12$; $p > 0.05$), with no interactions between HWT and storage period (skin ($F = 0.29$; $df = 5, 24$; $p > 0.05$). At 1 day of storage, 'Fuyu' persimmons treated at 44 ºC for 60 and 90 min exhibited a longer perforation distance (2.47 and 2.65 mm, respectively) than fruit from other treatments, which were similar to the control (Table 8). At 7 days of storage, all HWT fruit showed similar perforation distances to that of the control ($f = 2.76$; $df = 5, 12$; $p > 0.05$).

All parameters of skin color (L, a, b) were similar for the HWT fruit and the control (Table 9) for each storage period. No significant interaction was found between the HWT and both storage periods. However, considering the storage period alone (regardless of HWT), all color parameters were significantly lower at 7 days of storage, with reductions of 17.6%, 30.3%, and 56.4% for the color variables *L*, *a,* and *b*, respectively.

No differences for TA were detected among HWT fruit stored for 1 day ($F = 0.59$; $df = 5, 12$; $p > 0.05$) and 7 days ($F = 2.51$; $df = 5, 12$; $p > 0.05$) (Table 10). The ºBrix of persimmons stored for 1 day ($F = 0.38$; $df = 5, 12$; $p > 0.05$) and 7 days ($F = 1.60$; $df = 5, 12$; $p > 0.05$) was not significantly different. Furthermore, there was no interaction between the HWT and storage period for TA ($F = 70.37$; $df = 5, 24$; $p > 0.05$).

At 1 day of storage, persimmons treated at 44 ºC for 90 min, 46 ºC for 30 min, and 48 ºC for 20 min showed significantly higher pH values than the control. By contrast, at 7 days of storage, persimmons treated at 46 ºC for 20 and 30 min or 48 ºC for 20 min exhibited lower pH values than the control (Table 10). Considering the storage period alone (regardless of HWT), a significant decrease was observed at 7 days of storage for both pH ($F = 30.13$; $df = 1, 24$; $p < 0.05$) and ºBrix ($F = 30.13$; $df = 1, 24$; $p < 0.05$), which were reduced by 3.21% and 5.84%, respectively.

4. DISCUSSION

Heating techniques are used to increase the temperature of a commodity above the thermal limit of the insect species of interest [11]. Lethality is a function of both temperature and time [27]. The mortality rates of fruit flies submerged in hot water are not linear with exposure times [28]. Thermal tolerance varies among fruit fly species, populations, developmental stages, ages, fruit species, and variety [9, 11, 29, 30, 31, 32] and includes evolutionary factors [33].

Determining the most thermotolerant species and life stage is essential during post-harvest treatment studies [15]. Commodity tolerance to HWT must be considered at both the laboratory and commercial scale [34] and dose-response

testing is necessary to predict the heat treatment conditions during the large-scale phase [11]. In confirmatory tests, Hall et al. [35] concluded that 45 ºC for 40 min resulted in no survivors in zucchini (*Cucurbita pepo* L.) fruits infested with eggs of *Bactrocera cucumis* (French).

Our HWT results demonstrate the importance of the combined choice of temperature and period of exposure for insect disinfestation purposes. Even an immersion time of 90 min *in vitro* did not completely prevent the pupation of *C. capitata* eggs. By contrast, larvae exposed to 41 ºC and 42 ºC for short immersion times (15 min), or 48 ºC, reached the quarantine criteria for *A. fraterculus* (no emergence from treated third-instar larvae). The higher temperature during HWT probably increased the detrimental effect of oxygen depletion in the short exposure time, affecting other metabolic processes and changing of transcription of genes in the insects [28, 36].

In general, treatment of both immature stages (eggs and larvae) of *C. capitata in vitro* at 46ºC or higher for 20–40 min prevented the pupation and emergence of adults. A similar phenomenon occurred during *in vitro* tests of *A. fraterculus*. Vieira et al. [37] used *C. capitata* eggs and concluded that *in vitro* temperatures equal to or above 46 ºC for 20 min or more resulted in 100% mortality.

Jang [19] concluded that the eggs of *C. capitata* became inviable at temperatures above 46ºC and that they were more thermotolerant than eggs of *Bactrocera dorsalis* (Hendel) and *B. cucurbitae* (Coquillett). No significant differences were detected between young or old eggs of *C. capitata* submitted to hot forced air [38]. Sharp and Chew [39] estimated that exposure times of 24.8 min at 43 °C, 8.3 min at 46.1 ºC, 2.0 min at 48.9 ºC, 1.2 min at 51.7 ºC, and 0.9 min at 54.4 ºC were necessary to cause 99.9968% mortality of eggs of *Anastrepha suspensa* (Loew). Jang et al. [18] observed that exposure to 46 ºC for approximately 40 min was required to cause mortality of *B. latifrons* (Hendel) eggs, whereas *B. cucurbitae, B. dorsalis,* and *C. capitata* required an average of 10 min. Nascimento et al. [40] reported small differences in the time-mortality relationships of eggs and larvae of *C. capitata, A. fraterculus,* and *A. obliqua* subjected to HWT.

For each treatment (temperature vs. time), there were practically no differences between the number of pupae and adults for the respective fruit fly species. Pupation is probably more affected than the emergence of adults from immatures subjected to HWT. Verghese et al. [32] performed an artificial infestation of 'Totapuri' mangoes with *B. dorsalis* and reported complete mortality *in situ* of eggs and larvae by HWT at 48 ºC for 45 min.

Based on $LT_{99,}$ third-instar larvae of *C. capitata* were slightly more thermosensitive than those of *A. fraterculus* under *in vitro* conditions. The same effect was achieved during fruit tests, resulting in larger numbers of pupae and adults of *A. fraterculus* for the same temperatures and exposure times. A similar LT_{90} (27.70 min) as that obtained here for *A. fraterculus* was mentioned by Neven and Rehfield-Ray [41], who treated third-instar larvae of *Rhagoletis indifferens* Curran at 47 ºC. The third-instar larvae of *A. fraterculus* tested here were more thermoresistant than those of *A. suspensa* [39]. Thus, even among species belonging to the *fraterculus* group, there are differences in thermotolerance. Thomas and Mangan [42] determined that the third-instar larvae of *A. ludens* were more thermoresistant than those of *A. obliqua.*

Our estimated LT_{99} at 46 ºC and 48 ºC for both fruit flies after treatment of third-instar larvae were higher than the values obtained for the same temperatures by Waddell et al. [20] for *Bactrocera melanotus* (Coquillett) and *B. xanthodes* (Broun). By comparing the survival of *C. capitata* at the different temperatures and exposure times in the present study, we found that the third-instar larvae were slightly more thermosensitive than eggs *in vitro*. These results agree with *C. capitata in vitro* studies conducted by Sharp and Chew [39] and Gazit et al. [43]. Eggs of *A. fraterculus* were more susceptible than eggs of *C. capitata* to HWT *in vitro* because 20.81 min was the LT_{99} at 46 ºC for larval hatching.

One of the most important effects of temperature is its impact on enzymes and their function in modulating metabolites [44]. In this study, only third-instar larvae treated at temperatures above 42 ºC *in vitro* showed a reduction in the number of pupae for both fruit fly species studied. For the two species, 42 ºC seems to be the threshold temperature that causes undesirable metabolic changes, impairing the normal development of immature stages. Hallman et al. [45] estimated the LT_{99} for third-instar larvae of *Bactrocera*

invadens Drew, Tsuruta & White shorter immersion time (50 minutes) at 44 ºC than than the one in this study (≥ 90 min). Thomas and Mangan [46] reported that, to prevent the pupation of *A. ludens*, it is necessary to treat third-instar larvae for 40 min at 45 °C. Lopes et al. [47] treated mandarins infested with second-instar larvae of *C. capitata* and observed 100% larval mortality after HWT at 46 ºC for 32 min and 50 ºC for 21 min.

Thermal energy delivered to the interior of the fruit is affected by the fruit size, heating medium, and heating method [48]. Here, a long treatment time (90 min) was required to achieve the disinfestation effectiveness criteria in 'Fuyu' persimmon subjected to HWT, probably because a long heating time was necessary to transfer the desired temperature to the center of the fruit, where the larvae were located.

'Fuyu' persimmons infested with eggs and third-instar larvae and treated at 44 ºC for 90 min reached the criteria of no pupation or emergence of adults for both fruit flies. Vieira [49] applied HWT to 'Kumagai' guavas infested with *C. capitata* eggs and found that 47 ºC for 36 min prevented pupation. The HWT immersion time required for *A. fraterculus* larvae in 'Fuyu' persimmons (90 min at 44 ºC) was longer than that needed for guavas infested with *A. suspensa* (32.7 min at 46 °C) [50] but shorter than that required for *A. ludens* in 'Ataulfo' mangoes (110 min at 46 ºC) [51].

The difference in firmness observed between the first and seventh day of storage after HWT reveals that the treated persimmons were similar to the control under a normal ripening process. HWT can be used to delay the loss of firmness in 'Fuyu' persimmons, maintaining their commercial quality [52]. According to Lima et al. [53], during the growth and ripening of 'Fuyu' persimmon, fruit firmness decreases considerably, particularly during the last few days of the developmental period. This effect was more pronounced in astringent persimmons treated for 20 or 30 min at 45 ºC, which exhibited substantial softening [54]. The decline in firmness is due to the release of water and enzymatic activity that disrupts the cell wall [55] and this parameter may vary according to the fruit size [56].

No internal or external damage was observed in 'Fuyu' persimmons exposed to 47 ºC for 90 and 120 min. Furthermore, HWT reduced the severity of chilling injury during cold storage [52]. Ozdemir et al. [57] removed astringency from persimmon by applying HWT at 50 ºC for 5 h, followed by 7 days of storage.

HWT at 44 ºC with longer immersion times (60 and 90 min) and 46 ºC and 48 ºC for shorter exposure times (20 and 30 min) did not result in significant effects in the sugar concentration or skin color of persimmon. A similar observation was reported by Jabbar et al. [58] for mangoes treated at 48 °C for 60 min. In a study carried out with hot air treatment at similar temperatures, Woolf et al. [59] concluded that the use of heat did not affect the levels of soluble solids in 'Fuyu' persimmons, which remained in the range of 14–15%.

Regarding taste and flavor, TA did not differ among treatments after 1 and 7 days of storage. Persimmons treated at 46 ºC and 48 ºC showed a lower pH compared to fruits treated at 44 ºC and the control. Ozer et al. [60] reported that 'Hachiya' persimmons did not show significant differences in pH values after treatment. Lay-Yee et al. [52] did not detect changes in SST after HWT at 47 ºC for 90 and 120 min. The TA, TSS, and pH of the juice of mangoes treated at 48 ºC for 60 min after harvesting were similar to those of the control [55]. The HWT of 'Ataulfo' mangoes treated at 46.1 ºC for 95 min resulted in increased pH and TSS but firmness and acidity were similar to those of the control [61]. Higher TSS is crucial for the ripening process and the acceptance of the fruit in the market.

HWT with temperatures up to 50 ºC and no more than 30 min resulted in no apparent injury to bananas [34]. No significant differences between HWT (41.6 ºC for 72.63 min) and untreated 'Tommy Atkins' mangoes were observed regarding fruit firmness, pH, TSS, and TA 11 days post-treatment [9]. The same parameters were not affected in 'Apple' mangoes treated at 46.1 ºC for up to 84 min [62], whereas guavas tolerated 46.1ºC for 35 min [50].

5. CONCLUSION

- *C. capitata* eggs treated *in vitro* at temperatures ≥ 46 ºC for 30 min produced no pupae.
- *A. fraterculus* eggs treated *in vitro* at 46 ºC for 20 min exhibited no larval hatching.
- No pupae were obtained from third-instar larvae of both fruit fly species treated at 44 ºC for 60 min.

- Infested 'Fuyu' persimmons treated at 44 ºC for 90 min showed no emergence of adults of *C. capitata* or *A. fraterculus*.
- Except for pH, in general, 'Fuyu' persimmons subjected to HWT exhibited no alterations in skin and pulp texture, skin coloration, titratable total acidity, and total soluble solids.
- HWT showed potential as a quarantine treatment for the export of 'Fuyu' persimmons.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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